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GlcNAcstatin

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GlcNAcstatin - a picomolar, selective *O*-GlcNAcase inhibitor that modulates intracellular *O*-GlcNAcylation levels

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Supporting Information

Determination of the bOGA-GlcNAcstatin complex structure.

bOGA (NagJ from *Clostridium perfringens*) was expressed and purified as described previously [1]. Pure bOGA protein was spin concentrated to approximately 25 mg/ml. Vapour diffusion crystallisation experiments were set up by mixing 1 µl of protein, 1 µl of mother liquor (0.2 M ammonium sulfate, 0.1 M sodium cacodylate pH 6.5 and 30 % PEG 8000) and 0.25 µl of 40 % v/v γ -butyrolactone. Rod-shaped crystals appeared after 4 days growing to a maximum size of approximately 0.3 x 0.1 x 0.1 mm. Solid GlcNAcstatin was added to the drop for 20 min, after precipitant was removed from the drop. The crystal was cryoprotected by a 5 s immersion in a solution containing 0.17 M ammonium sulfate, 0.085 M sodium cacodylate, pH 6.5, 25.5% PEG 8000 and 15% glycerol, and then frozen in liquid nitrogen. Data were collected on beamline ID14-4 at the European Synchrotron Radiation Facility (Table I).

Table I

Details of data collection and structure refinement. Values between brackets are for the highest resolution shell. All measured data were included in structure refinement. The space group was $I2_12_12_1$.

| | bOGA-GlcNAcstatin |
|---------------------------------|----------------------------------|
| Unit cell (Å) | a=130.25 b=149.92 c=152.57 |
| Resolution range (Å) | 20.00-2.25 (2.33-2.25) |
| # Observed reflections | 253208 (25373) |
| # Unique reflections | 67921 (6729) |
| Redundancy | 3.7 (3.8) |
| I/ σ I | 12.8 (2.8) |
| Completeness (%) | 99.9 (100.0) |
| R _{merge} | 0.071 (0.535) |
| # Protein residues | 1170 |
| # Water molecules | 730 |
| R, R _{free} | 0.202, 0.249 |
| RMSD from ideal geometry | |
| bonds (Å) | 0.01 |
| angles (°) | 1.3 |
| B-factor RMSD (Å ²) | |
| (backbone bonds) | 0.57 |
| (Å ²) | |
| protein | 46.2 |
| inhibitor | 34.2 |
| solvent | 49.5 |

Refinement was initiated from the native structure [1], immediately revealing well defined $|F_o| - |F_c|$, ϕ_{calc} electron density for the inhibitor, which was built in with the help of PRODRG [2] generated inhibitor structure and topology. Further model building with COOT [3]) and refinement with REFMAC [4] then yielded the final model with statistics shown in Table I. The coordinates of the complex have been deposited with the PDB (entry 2J62).

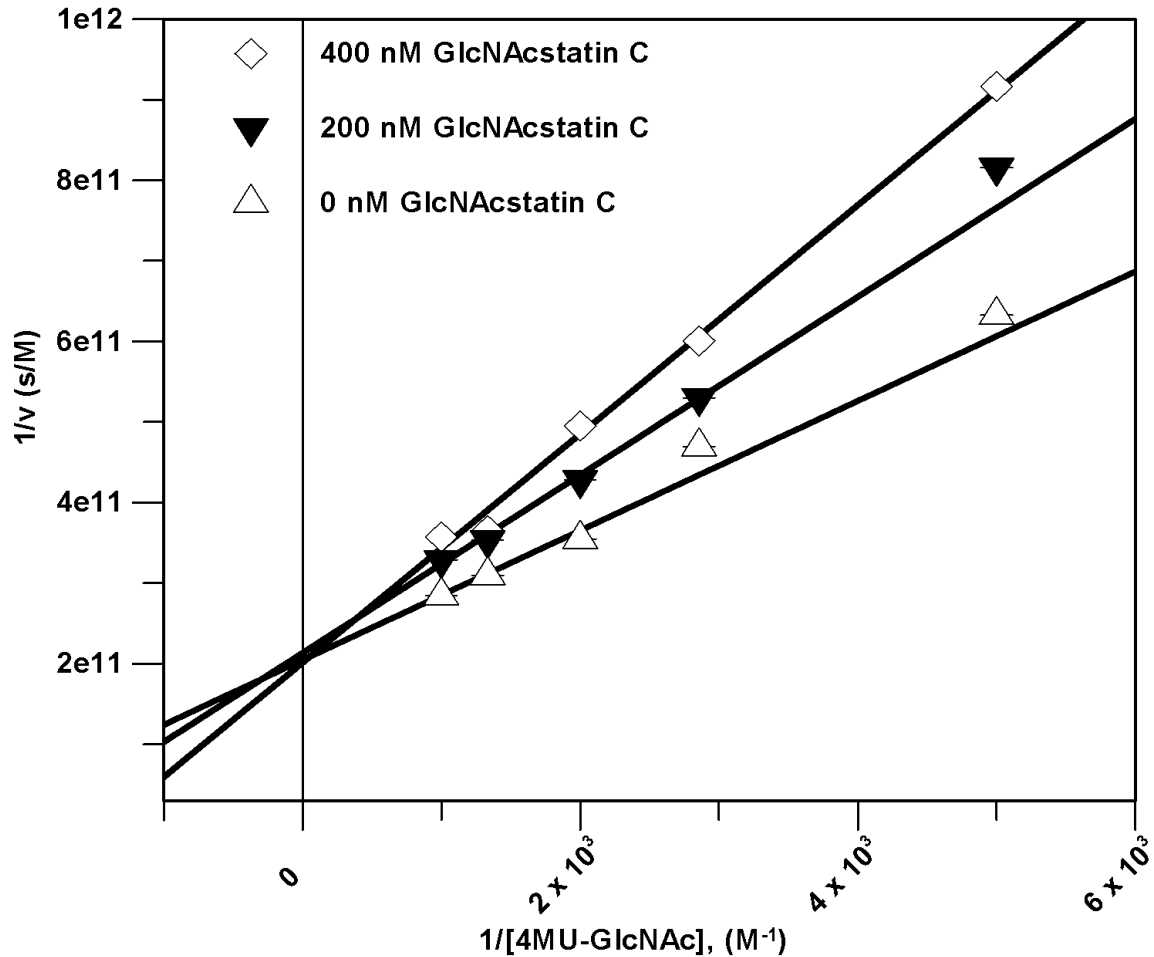
Enzymology

Steady state kinetics of bOGA were determined using the fluorogenic substrate 4-methylumbelliferyl- *N*-acetyl- β -D-glucosaminide (4MU-NAG; Sigma). Standard reaction mixtures (50 μ l) contained 2 pM bOGA in 50 mM citric acid, 125 mM NaHPO₄, 0.1 mg/ml BSA, and 1.5 - 25 μ M of substrate in water. The reaction mixture was incubated

for 466 min at 20 °C (RT). The reaction was stopped by the addition of a 2-fold excess (100 µl) of 3 M glycine-NaOH, pH 10.3. The fluorescence of the released 4-methylumbelliferone was quantified using a FLX 800 Microplate Fluorescence Reader (Bio-Tek), with excitation and emission wavelengths of 360 and 460 nm, respectively. The production of 4-methylumbelliferone was linear with time for the incubation period used, and less than 10% of the available substrate was hydrolysed. Experiments were performed in triplicate; all of the spectra were corrected for the background emission from the buffer and the protein. Michaelis-Menten parameters were obtained by fitting the fluorescence intensity with GraFit [5].

Determination of the GlcNAcstatin K_i was performed by steady-state kinetics in the presence of different concentrations (0, 35, 70, 140 pM) of the inhibitor (which was dissolved in 25% DMSO/75% water and then diluted at least 5×10^8 fold). The mode of inhibition was visually verified by the Lineweaver-Burk plot (see Fig. 1 in the main text), and the K_i determined by fitting all fluorescence intensity data to the standard equation for competitive inhibition in GraFit [5]. Using this approach, the following parameters were determined from the data shown: $K_i = 4.6 \pm 0.2$ pM, $K_m = 2.6 \pm 0.1$ µM, $k_{cat} = 8.1 \pm 0.5$ s⁻¹, with the Michaelis-Menten parameters close to those published previously [1].

Steady state kinetics of hexosaminidases A and B (purchased from Sigma, catalogue number A6152), were determined using the fluorogenic substrate 4MU-GlcNAc substrate described above, using the same standard reaction mixture, with the following changes: 5×10^{-6} units / ml enzyme mixture was used with a varying substrate concentration between 200 and 1000 µM. Determination of the GlcNAcstatin K_i for placental hexosaminidases was performed by steady-state kinetics in the presence of different concentrations (0, 200, 400 nM) of the inhibitor, and interpreted as for bOGA, with the data shown see Figure below. The following parameters were determined from the data shown: $K_i = 0.52 \pm 0.07$ µM, $K_m = 400 \pm 40$ µM.



Cell culture and immunoblotting

HEK 293 human embryonic kidney cells and SH-SY5Y neuroblastomas were maintained using standard tissue culture techniques. HEK 293 were exposed to OGA inhibitors for 12 h at the given concentrations. Cell lysates were prepared in 50 mM Tris buffer (pH 7.4) containing 0.27 M sucrose, 1 mM Na-orthovanadate, 1 mM EDTA, 1 mM EGTA, 10 mM Na-glycerophosphate, 50 mM NaF, 1 % Triton-X100, 0.1 % β -mercaptoethanol and protease inhibitors. Total Protein concentrations were estimated by Bradford assay. bOGA activity against SH-SY5Y lysates was determined as previously described [1].

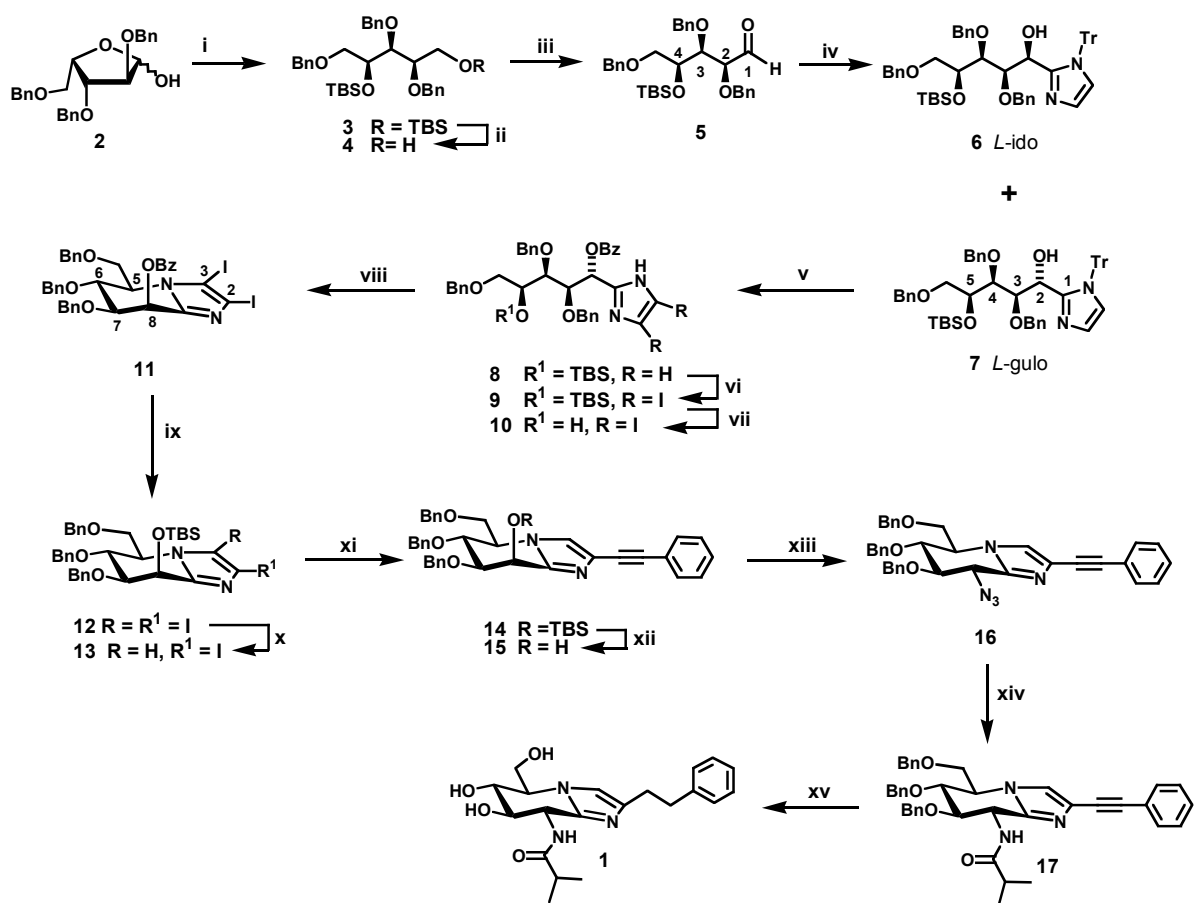
10 μ g total protein were separated electrophoretically on 3-8% Tris-Acetate polyacrylamide gels. Following transfer onto nitrocellulose membrane, non-specific binding sites were blocked with bovine serum albumine. *O*-GlcNAc levels were determined with the CTD 110.6 *O*-GlcNAc specific mouse monoclonal antibody, using a HRP-linked secondary antibody and the enhanced chemiluminescence detection method. Membranes were subsequently stripped and re-probed for β -tubulin to ensure equal loading.

Synthesis

General

All reactions were performed in oven-dried glassware under an inert atmosphere (argon) unless noted otherwise. Dichloromethane, toluene and acetonitrile were distilled from CaH_2 prior to use. Tetrahydrofuran was distilled from sodium-benzophenone prior to use. N,N-dimethylformamide, pyridine, triethylamine were anhydrous grade solvents from Fluka. Ethyl acetate (EA), petroleum spirit 40-60° (PE), diethyl ether (EE), and chloroform were laboratory grade solvents. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 aluminium plates (0.25mm). Compounds were visualized by UV light, or by dipping the plate into acidic potassium permanganate aqueous solution followed by washing out the excess of the reagent, or by charring the plate at *ca.* 300°C after dipping in one of the following solutions: 15% sulphuric acid in water-ethanol, 5% phosphomolybdic acid in ethanol, orcinol in acidic ethanol. Flash column chromatography was performed on Merck Kieselgel 60 (230-400 mesh). NMR spectra were recorded on a Bruker DPX300 or Bruker AVANCE II 500 spectrometer in CDCl_3 unless otherwise stated. Chemical shifts are referenced to internal CDCl_3 (7.26 ppm ^1H , 77.0 ppm ^{13}C). Splitting patterns of spectral multiplets are indicated as s, singlet; d, doublet; bs broad singlet; bd broad dublet; t triplet; quint quintet for ^1H NMR data. Signals were assigned by means of DEPT, COSY, HSQC, and HMBC spectra. High-resolution mass spectra (HRMS) were obtained on a microTOF Bruker Daltonics instrument. Optical rotations were measured in chloroform on a Perkin Elmer 343 polarimeter.

Overview of the synthetic approach to GlcNAcstatin



Reagents and conditions: (i) a) NaBH₄, MeOH, 0° to RT, 1h; b) TBSCl, ImH, DMF, +55°C, overnight, 95% overall; (ii) TFA, H₂O, CHCl₃, 15 min, RT, 65%; (iii) (COCl)₂, DMSO, Et₃N, DCM, -60°C, 93%; (iv) N-trityl imidazole, BuLi, THF, -78°C, 70% of a 3:1 mixture of **7** and **6**; (v) a) TFA, DCM then Et₃SiH, 25 min, RT; b) BzCl, Py, DMAP cat., 30 min, RT, 91% overall; (vi) *N*-Iodosuccinimide, MeCN, 6h, RT, 90%; (vii) HCl, 1,4-dioxane, 2h, RT, quant.; (viii) Tf₂O, Py, DCM, -15°C to RT, 12 h then +50°C, 1h, 89%; (ix) (a) MeONa, MeOH, DCM, 10 min, RT; (b) TBSOTf, *i*-Pr₂NEt, DCM, -15°C to RT; 91% overall; (x) EtMgBr, THF, 20 min, 0°, 93%; (xi) C₆H₅CCH, Pd(PPh₃)₄, CuI, Et₃N, DMF, 12h, +80°C, 96%; (xii) TBAF, THF, 1h, RT, 83%; (xiii) DPPA, DBU, toluene-THF, 12h, RT, 90%; (xiv) PPh₃, THF-H₂O, 3h, +60°C, then (*i*-PrCO)₂O, Et₃N, 1h RT, 95%; (xv) Pd(OH)₂, H₂ 14.5 psi, AcOH, 5h RT, 60%.

Experimental procedures

Compound 3. To a solution of **2** [1, 2] (6.25 g, 14.9 mmol) in MeOH (100 ml) sodium borohydride (NaBH_4 ; 0.56 g, 14.8 mmol) was added at 0°C (ice-bath). The resulting slurry was stirred for 1h while the reaction was allowed to warm up to the RT. The remaining sodium borohydride was quenched by careful addition of glacial acetic acid (3.4 ml, 59.24 mmol) and the reaction was evaporated to dryness. The residue was dissolved in DCM and washed with a mixture of saturated NaHCO_3 solution and brine. The aqueous layer was extracted with DCM once more. The combined organic layer was dried and evaporated. The residue was dissolved in DMF (90 ml) and treated with *tert*-butyl(chloro)dimethylsilane (TBSCl; 5.42 g, 36 mmol) in the presence of imidazole (ImH; 4.9 g, 72 mmol) for 16h at $+50^\circ\text{C}$. The reaction was cooled down and quenched by the addition of MeOH (5 ml). The bulk of DMF was removed in vacuum. The residue was dissolved in ethyl acetate and washed with water. The aqueous layer was extracted with ethyl acetate once more. The combined organic layer was dried and evaporated. The residue was purified by flash column chromatography in PE-EE gradient 1 \rightarrow 10% to give 9.24 g (14.2 mmol, 95%) of the target product ($R_f = 0.37$ PE-EE 10%).

$[\alpha]_D = -21.1^\circ$ c 1.0 CHCl_3

HRMS: Positive mode, $m/z = 673.3766$; expected for $\text{C}_{38}\text{H}_{58}\text{NaO}_5\text{Si}_2$ $[\text{M}+\text{Na}]^+ = 673.3720$.

δ_{H} (500 MHz): -0.01 and 0.002 and 0.04 (12H, 4 \times s, $-\text{Si}(\text{CH}_3)\text{C}(\text{CH}_3)_3$), 0.88 (18H, 2 \times s, $-\text{Si}(\text{CH}_3)\text{C}(\text{CH}_3)_3$), 3.43 (1H, dd, $J_{4,5} = 7.2$ Hz, $J_{5a,5b} = 10.3$ Hz, H-5a), 3.6 (1H, dd, $J_{1,2} = 5.6$ Hz, $J_{1a,1b} = 10$ Hz, H-1a), 3.64 (1H, dd, $J = 5.6$ Hz, $J = 3.2$ Hz, H-3), 3.68-3.75 (3H, m), 4.02 (1H, m, H-4), 4.43 (2H, AB spectrum, $J_{\text{gem}} = 12$ Hz, $\text{PhCH}_2\text{O}-$), 4.54 and 4.63 (2H, AB spectrum, $J_{\text{gem}} = 12$ Hz, $\text{PhCH}_2\text{O}-$), 4.65 (2H, AB spectrum, $J_{\text{gem}} = 11.5$ Hz, $\text{PhCH}_2\text{O}-$), 7.23 -7.33 (15H, m, 3 \times $\text{PhCH}_2\text{O}-$).

δ_{C} (125 MHz): -5.4, -5.3, -4.8, -4.3, 18.2, 18.24, 26, 62.4, 72.18, 72.5, 73.1, 73.4, 74.1, 76.9, 77.1, 77.4, 78.9, 127.4, 127.6, 127.6, 128.3, 138.7.

Compound 4: To a solution of **3** (4.4 g, 6.76 mmol) in chloroform (70 ml) aqueous trifluoroacetic acid (TFA-H₂O 10:1 v/v; 7 ml) was added at RT. The resulting solution was kept for 10 min, diluted with toluene (70 ml) and evaporated to dryness. The residue was dissolved in DCM and washed with a mixture of saturated NaHCO₃ solution and brine. The aqueous layer was extracted with DCM once more. The combined organic layer was dried and evaporated. The residue was purified by flash column chromatography in PE-EE 10% and then in PE-EA gradient 10 → 20% to give 1.16 g (1.78 mmol, 26%) of the unchanged starting material and 2.24 g (4.17 mmol, 62%) of the target product ($R_f = 0.35$ PE-EA 20%) (83% based on the recovered starting material).

$[\alpha]_D = -8.5^\circ$ c 1.1 CHCl₃

HRMS: Positive mode, $m/z = 559.2902$; expected for C₃₂H₄₄NaO₅Si $[M+Na]^+ = 559.2856$.

δ_H (300 MHz): 0.04 and 0.0 (6H, 2×s, -Si(CH₃)C(CH₃)₃), 0.82 (9H, s, -Si(CH₃)C(CH₃)₃), 2.52 (1H, t, $J = 6.5$ Hz, 1-OH), 3.48 (1H, dd, $J_{4,5} = 7$ Hz, $J_{5a,5b} = 10$ Hz, H-5a), 3.5-3.7 (5H, m), 4 (1H, ddd, $J = 3$ Hz, $J = 4.3$ Hz, H-4), 4.42 (2H, s, PhCH₂O-), 4.55 and 4.62 (2H, AB spectrum, $J_{gem} = 11.5$ Hz, PhCH₂O-), 4.61 and 4.68 (2H, AB spectrum, $J_{gem} = 11.7$ Hz, PhCH₂O-), 7.2 -7.3 (15H, m, 3×PhCH₂O-).

δ_C (75 MHz): -4.9, -4.4, 18.15, 25.9, 62.24, 71.7, 72.4, 73.3, 73.4, 74.2, 79.9, 81.2, 127.5, 127.6, 127.7, 127.8, 128.1, 128.16, 128.33, 128.44, 138.4, 138.45.

Compound 5: To a solution of oxalyl chloride (1.12 ml, 12.9 mmol) in DCM (70 ml) a solution of dimethyl sulfoxide (1.83 ml, 12.9 mmol) in DCM (3 ml) was added dropwise at -60°C. The resulting solution was stirred for 15 min, and then a solution of **4** (4.62 g, 8.6 mmol) in DCM (20 ml) was added via cannula. The resulting solution was stirred at -60°C for 1 h and then triethylamine (5.4 ml, 38.7 mmol) was added dropwise. The resulting slurry was stirred for 10 min and then warmed up to 0°C and quenched by the addition of 1M HCl solution (40 ml). The layers were separated and the aqueous layer was extracted with DCM once more. The combined organic layer was washed successively with water and a mixture of saturated NaHCO₃ solution and brine, dried and

evaporated. The residue was purified by flash column chromatography in PE-EA 10 % to give 4.27 g (8 mmol, 93%) of the target product (R_f = 0.25-0.4 PE-EA 10%).

$[\alpha]_D = -16.3^\circ$ c 1.04 CHCl_3

HRMS: Positive mode, m/z = 535.2854; expected for $\text{C}_{32}\text{H}_{43}\text{O}_5\text{Si}$ $[\text{M}+\text{H}]^+ = 535.2880$.

δ_H (500 MHz): 0.0 and 0.04 (6H, 2 \times s, -Si(CH₃)C(CH₃)₃), 0.89 (9H, s, -Si(CH₃)C(CH₃)₃), 3.5 (1H, dd, $J_{5a,4} = 7$ Hz, $J_{5a,5b} = 10$ Hz, H-5a), 3.74 (1H, dd, $J_{5b,4} = 7$ Hz, $J_{5a,5b} = 10$ Hz, H-5b), 3.84 (1H, dd, $J_{3,4} = 7$ Hz, $J_{3,2} = 10$ Hz, H-3), 4.02 (1H, d, H-2), 4.05 (1H, ddd, H-4), 4.47 (2H, s, PhCH₂O-), 4.52 and 4.68 (2H, AB spectrum, $J_{gem} = 11.5$ Hz, PhCH₂O-), 4.61 (2H, s, PhCH₂O-), 7.2 -7.35 (15H, m, 3 \times PhCH₂O-), 9.65 (1H, s, H-1).

δ_C (125 MHz): -4.8, -4.5, 18.1, 25.9, 71.5 (C-4 and C-5), 73.2, 74.1, 80.2 (C-3), 83.2 (C-2), 127.6, 127.7, 128, 128.1, 128.2, 128.4, 128.44, 128.5, 137.2, 137.8, 138.3, 202.3 (C-1).

Compounds 6 and 7: To a solution of N-trityl imidazole (2.67 g, 8.6 mmol) in THF (85 ml) cooled to -20°C a stock 1.6 M solution n-butyl lithium in hexanes (5.13 ml, 8.2 mmol) was added dropwise. The resulting wine red coloured solution was allowed to warm up to RT and stirred for 30 min. The solution was then cooled to -78°C and a solution of aldehyde (4.17 g, 7.8 mmol) in THF (15ml) was added dropwise. The resulting solution was stirred for 30 min and then allowed to warm to RT in 30 min. The reaction was quenched by addition of ammonium chloride solution and extracted with ethyl acetate. The aqueous layer was extracted with ethyl acetate once more. The combined organic layer was dried and evaporated. The residue was purified by flash column chromatography in PE-EA gradient 10→20→30% to give 1.18 g (1.4 mmol, 18%) of **6** (R_f = 0.55 PE-EA 20%) and 3.48 g (4.13 mmol, 53%) of **7** (R_f = 0.4 PE-EA 20%).

6: $[\alpha]_D = -10.5^\circ$ c 1.24 CHCl_3

HRMS: Positive mode, m/z = 845.4344; expected for $\text{C}_{54}\text{H}_{61}\text{N}_2\text{O}_5\text{Si}$ $[\text{M}+\text{H}]^+ = 845.4350$.

δ_H (500 MHz): 0.0 and 0.02 (6H, 2 \times s, -Si(CH₃)C(CH₃)₃), 0.89 (9H, s, -Si(CH₃)C(CH₃)₃), 2.94 (1H, dd, $J_{4,5}$ = 6.5 Hz, $J_{4,3}$ = 4.1 Hz, H-4), 3.1 (1H, bs, 2-OH), 3.14 (1H, dd, $J_{6a,5}$ = 5.4, $J_{6a,6b}$ = 10.2 Hz, H-6a), 3.37 (1H, dd, $J_{6b,5}$ = 3 Hz, H-6b), 3.8 (1H, dd, $J_{3,2}$ = 3.2 Hz, H-3), 3.93 (1H, ddd, H-5), 3.97 and 4.41 (2H, AB spectrum, J_{gem} = 11.5 Hz, PhCH₂O-), 4.3 (1H, bs, H-2), 4.33 (2H, s, PhCH₂O-), 4.71 and 4.93 (2H, AB spectrum, J_{gem} = 11.2 Hz, PhCH₂O-), 6.78 (1H, m), 7.02 (2H, m), 7.13-7.4 (28H, m).

δ_C (125 MHz): -4.4, 18.2, 26.1, 66.6 (C-2), 72.8 (C-6), 72.9 (C-5), 73.2, 73.8, 74.4, 75.3, 78.8 (C-3), 81.7 (C-4), 122.2, 126.08, 127.06, 127.3, 127.4, 127.8, 128.1, 128.3, 128.5, 130, 138.6, 138.8, 138.9, 142.9, 150.7.

7: $[\alpha]_D = -67.5^\circ$ c 1.34 CHCl₃

HRMS: Positive mode, m/z = 845.4344; expected for C₅₄H₆₁N₂O₅Si [M+H]⁺ = 845.4350.

δ_H (500 MHz): -0.09 and 0.0 (6H, 2 \times s, -Si(CH₃)C(CH₃)₃), 0.82 (9H, s, -Si(CH₃)C(CH₃)₃), 3.52 (1H, dd, $J_{6a,5}$ = 6.5 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6a), 3.73 (1H, dd, $J_{4,5}$ = 7.3, $J_{4,3}$ = 2.6 Hz, H-4), 3.78 (1H, dd, $J_{6b,5}$ = 2.2 Hz, H-6b), 4.12 (1H, ddd, $J_{5,4}$ = 6.7 Hz, H-5), 4.24 (1H, dd, $J_{3,2}$ = 8.7 Hz, H-3), 4.27 and 4.57 (2H, AB spectrum, J_{gem} = 12.4 Hz, PhCH₂O-), 4.41 (2H, s, PhCH₂O-), 4.48 and 4.6 (2H, AB spectrum, J_{gem} = 10.7 Hz, PhCH₂O-), 4.5 (1H, dd, $J_{2,OH}$ = 4 Hz, H-2), 6.79 (1H, m), 6.85-7.4 (31H, m).

δ_C (125 MHz): -4.7, 18.2, 26, 65.9 (C-2), 72.6 (C-5), 73.1, 73.2 (C-6), 73.8, 75.5, 79.6 (C-4), 80.13 (C-3), 122.3, 125.9, 126.17, 126.5, 127, 127.2, 127.6, 127.8, 127.9, 128.1, 128.4, 128.6, 130, 130.2, 138.7, 139, 139.3, 141, 142.5, 150.2 (C-1).

Compound 8: To a solution of **7** (1.13 g, 1.34 mmol) in chloroform (CHCl₃; 35 ml) trifluoroacetic acid (11 ml) was added at RT to give bright yellow solution. The reaction was kept for 25 min at RT and quenched by addition of an excess of triethylsilane (2ml, 12.5 mmol) (yellow colouration disappeared). The reaction was diluted with toluene (50 ml) and evaporated. The residue was taken up into DCM and washed with a mixture of NaHCO₃ solution and brine. The aqueous layer was extracted with DCM once more. The

combined organic layer was dried and evaporated. The residue was dissolved in pyridine (15ml) and treated with benzoyl chloride (3 mmol) in the presence of catalytic amount of 4-*N,N*-dimethylaminopyridine. After 30 min the reaction was quenched by addition of water and stirred for 20 min. The bulk of pyridine was evaporated in vacuum. The residue was dissolved in a mixture of methanol and triethylamine 4:1 (10 ml), kept for 30 min at RT and evaporated. The residue was purified by flash column chromatography in PE-EA gradient 10→20→25% to give 0.86 g (1.21 mmol, 91%) of the target product ($R_f = 0.35$ PE-EA 25%).

$[\alpha]_D = -3.9^\circ$ c 0.87 CHCl_3

HRMS: Positive mode, $m/z = 707.3481$; expected for $\text{C}_{42}\text{H}_{51}\text{N}_2\text{O}_6\text{Si}$ $[\text{M}+\text{H}]^+ = 707.3516$.

δ_H (500 MHz): 0.0 (6H, s, $-\text{Si}(\text{CH}_3)\text{C}(\text{CH}_3)_3$), 0.82 (9H, s, $-\text{Si}(\text{CH}_3)\text{C}(\text{CH}_3)_3$), 3.43 (1H, dd, $J_{6a,5} = 6.5$ Hz, $J_{6a,6b} = 10.1$ Hz, H-6a); 3.6 (1H, dd, $J_{4,3} = 5$ Hz, $J_{4,5} = 4.3$ Hz, H-4), 3.65 (1H, dd, $J_{6b,5} = 3.1$ Hz, H-6b), 4.1 (1H, ddd, H-5), 4.33 and 4.43 (2H, AB spectrum, $J_{\text{gem}} = 11.6$ Hz, $\text{PhCH}_2\text{O}-$), 4.36 (2H, s, $\text{PhCH}_2\text{O}-$), 4.39 and 4.46 (2H, AB spectrum, $J_{\text{gem}} = 10.8$ Hz, $\text{PhCH}_2\text{O}-$), 4.47 (1H, dd, $J_{3,2} = 5.3$ Hz, H-3), 6.16 (1H, d, H-2), 6.83 (1H, s, H-3'), 6.96-7.25 (16H, m, $3 \times \text{PhCH}_2\text{O}-$, H-2'), 7.3 (2H, m), 7.46 (1H, m), 7.9 (2H, m), 9.7 (1H, bs, NH).

δ_C (125 MHz): -4.8, -4.3, 18.2, 26, 69.9 (C-2), 71.7 (C-5), 72.15 (C-6), 73.1, 73.9, 74.9, 79.18 (C-3/C-4), 79.25 (C-3/C-4), 116.3, 127.4, 127.6, 127.8, 127.9, 128.4, 128.5, 129.6, 129.9, 133.2, 137.7, 137.8, 138.5, 144 (C-1), 165.3.

Compound 9: To a stirred solution of **8** (1.03 g, 1.46 mmol) in acetonitrile (15 ml) *N*-iodosuccinimide (0.818 g, 3.65 mmol) was added at RT. The reaction was further stirred for 12h in the dark. The reaction was diluted with DCM and washed with a mixture of 0.5M sodium thiosulphate solution and brine. The aqueous layer was extracted with DCM once more. The combined organic layer was dried and evaporated. The residue was purified by flash column chromatography in PE-EA gradient 5→10→15% to give 1.26 g (1.31 mmol, 90%) of the target product ($R_f = 0.36$ PE-EA 15%).

$[\alpha]_D = -5.6^\circ$ *c* 1.19 CHCl_3

HRMS: Positive mode, $m/z = 959.1440$; expected for $\text{C}_{42}\text{H}_{49}\text{I}_2\text{N}_2\text{O}_6\text{Si}$ $[\text{M}+\text{H}]^+ = 959.1449$.

δ_{H} (500 MHz): 0.0 and 0.02 (6H, 2×s, $-\text{Si}(\text{CH}_3)\text{C}(\text{CH}_3)_3$), 0.83 (9H, s, $-\text{Si}(\text{CH}_3)\text{C}(\text{CH}_3)_3$), 3.43 (1H, dd, $J_{6a,5} = 6.7$ Hz, $J_{6a,6b} = 10.3$ Hz, H-6a); 3.64 (1H, dd, $J_{4,3} = 3$ Hz, $J_{4,5} = 6$ Hz, H-4), 3.67 (1H, dd, $J_{6b,5} = 2.6$ Hz, H-6b), 4.12 (1H, ddd, H-5), 4.3 and 4.5 (2H, AB spectrum, $J_{\text{gem}} = 11$ Hz, $\text{PhCH}_2\text{O}-$), 4.38 and 4.43 (2H, AB spectrum, $J_{\text{gem}} = 12.3$ Hz, $\text{PhCH}_2\text{O}-$), 4.45 (2H, AB spectrum, $\text{PhCH}_2\text{O}-$), 4.41 (1H, H-3), 6.11 (1H, d, $J_{2,3} = 4.5$, Hz, H-2), 7.0 (4H, m), 7.1-7.26 (11H, m), 7.33 (2H, m), 7.5 (1H, m), 7.9 (2H, m), 9.7 (1H, bs, NH).

δ_{C} (125 MHz): -4.7, -4.4, 18.2, 26, 70 (C-2), 71.7 (C-5), 72 (C-6), 73.2, 74, 74.9, 75.8, 79.1 (C-3), 79.6 (C-4), 95.1, 127.5, 127.7, 127.9, 128.1, 128.2, 128.4, 128.5, 128.6, 129.3, 130, 133.5, 137.3, 137.4, 138.4, 150 (C-1), 165.2.

Compound 10: To intensively stirred solution of **9** (1.24 g, 1.3 mmol) in 1,4-dioxane (15 ml) concentrated hydrochloric acid (1.2 ml) was added at RT. The reaction was further stirred for 2h, diluted with DCM and washed successively with water and a mixture of NaHCO_3 solution and brine. The aqueous layer was extracted with DCM once more. The combined organic layer was dried and evaporated. The residue was purified by flash column chromatography in PE-EA gradient 10→20→30% to give 1.06 g (1.26 mmol, 96%) of the target product ($R_f = 0.3$ PE-EA 30%).

$[\alpha]_D = -6.7^\circ$ *c* 0.94 CHCl_3

HRMS: Positive mode, $m/z = 845.0585$; expected for $\text{C}_{36}\text{H}_{35}\text{I}_2\text{N}_2\text{O}_6$ $[\text{M}+\text{H}]^+ = 845.0585$.

δ_{H} (300 MHz): 3.18 (1H, d, $J_{5,\text{OH}} = 6$ Hz, 5-OH); 3.56 (2H, m, H-6a, H-6b), 3.85 (1H, dd, $J_{4,3} = 6$ Hz, $J_{4,5} = 3$ Hz, H-4), 4.15 (1H, m, H-5), 4.5 (1H, dd, H-3), 4.51 (2H, AB spectrum, $\text{PhCH}_2\text{O}-$), 4.55 and 4.64 (2H, AB spectrum, $J_{\text{gem}} = 11$ Hz, $\text{PhCH}_2\text{O}-$), 4.68 (2H, AB spectrum, $\text{PhCH}_2\text{O}-$), 6.3 (1H, d, $J_{2,3} = 4$ Hz, H-2), 7.16-7.42 (17H, m), 7.58 (1H, m), 8 (2H, m), 10.1 (1H, bs, NH).

δ_C (75 MHz): 69.5 (C-5), 69.7 (C-2), 70.8 (C-6), 73.3, 74.9, 75, 76.2, 78.4 (C-4), 80 (C-3), 94.9, 127.7, 127.8, 127.9, 128.1, 128.2, 128.4, 128.6, 129.1, 130, 133.6, 137.4, 137.6, 137.9, 149.9 (C-1), 165.7.

Compound 11: To a solution of **10** (1.04 g, 1.23 mmol) and Py (0.41ml, 5.05 mmol) in DCM (15 ml) cooled to -15°C trifluoromethanesulfonic anhydride (0.62ml, 3.77mmol) was added dropwise. The reaction was allowed to warm-up to the RT and left stirred for 24 h. At this point TLC PE-EA 15% still showed the presence of the upper spot (presumably intermediate triflate) and the more polar major product. The reaction was heated for 1.5 h at 50°C to promote the disappearance of the upper spot. The reaction was cooled down, diluted with DCM and washed with a mixture of NaHCO_3 solution and brine. The aqueous layer was extracted with DCM once more. The combined organic layer was dried evaporated. The residue was purified by flash column chromatography in PE-EA gradient 10 \rightarrow 20 \rightarrow 30% to give 0.902 g (1.1 mmol, 89%) of the target product (R_f = 0.35 PE-EA 30%).

$[\alpha]_D = -101.6^\circ$ c 1.0 CHCl_3

HRMS: Positive mode, m/z = 827.0454; expected for $\text{C}_{36}\text{H}_{33}\text{I}_2\text{N}_2\text{O}_5$ $[\text{M}+\text{H}]^+ = 827.0479$.

δ_H (500 MHz): 3.74 (1H, m, $J_{5^*a,5} = 3.6$ Hz, $J_{5^*a,5^*b} = 9.8$ Hz, H-5*a), 4.0 (1H, m, $J_{5^*b,5} = 7.4$ Hz, H-5*b); 4.1 (1H, dd, $J_{7,6} = 7.5$ Hz, $J_{7,8} = 3.6$ Hz, H-7), 4.3 (1H, ddd, $J_{5,6} = 3.6$ Hz, H-5), 4.46 (2H, AB spectrum, $J_{\text{gem}} = 12.1$ Hz, $\text{PhCH}_2\text{O-}$), 4.54 and 4.75 (2H, AB spectrum, $J_{\text{gem}} = 11.7$ Hz, $\text{PhCH}_2\text{O-}$), 4.56 (1H, dd, H-6), 4.64 and 4.76 (2H, AB spectrum, $J_{\text{gem}} = 11.5$ Hz, $\text{PhCH}_2\text{O-}$), 6.65 (1H, d, H-8), 7.16-7.35 (15H, m), 7.38 (2H, m), 7.55 (1H, m), 8.06 (2H, m).

δ_C (125 MHz): 61.7 (C-5), 63.9 (C-8), 68.9 (C-5*), 72.4, 73.2, 73.8, 74 (C-6), 77.1 (C-7), 81.2 (C-2), 97.8 (C-3), 127.7, 128.02, 128.08, 128.22, 128.3, 128.4, 128.6, 129.6, 130.1, 133.3, 137.1, 137.4, 137.5, 146.7 (C-8a), 165.25.

Compound 12: To a solution of **11** (0.885 g, 1.07mmol) in DCM (15 ml) stock 25% solution of sodium methoxide (0.15 ml) was added at RT. After 10 min the reaction was quenched by addition of dry ice. After the reaction was attained RT it was diluted with DCM and washed with brine. The aqueous layer was extracted once more with DCM. The combined organic layer was dried and evaporated to dryness. The residue was dissolved in anhydrous DCM (12 ml) and treated with *tert*-butyldimethylsilyl trifluoromethanesulfonate (0.3 ml, 1.3 mmol) in the presence of diisopropylethylamine (0.3ml, 1.7mmol) at 0°C (ice bath). The reaction was allowed to warm-up to the RT and left stirred for 30 min. The reaction was quenched with MeOH (1 ml) diluted with DCM and washed with a mixture of NaHCO₃ solution and brine. The aqueous layer was extracted with DCM once more. The combined organic layer was dried and evaporated. The residue was purified by flash column chromatography in PE-EE gradient 10→20% to give 0.822 g (0.98 mmol, 91%) of the target product (R_f = 0.4 PE-EE 20%).

$[\alpha]_D = -82.8^\circ$ c 0.83 CHCl₃

HRMS: Positive mode, m/z = 837.1047; expected for C₃₅H₄₃I₂N₂O₄Si $[M+H]^+$ = 837.1082.

δ_H (500 MHz): 0.0 and 0.13 (6H, 2×s, -Si(CH₃)C(CH₃)₃), 0.83 (9H, s, -Si(CH₃)C(CH₃)₃), 3.6 (1H, m, , $J_{5^*a,5} = 4.9$ Hz, $J_{5^*a,5^*b} = 9.2$ Hz, H-5^{*a}), 3.62 (1H, dd, $J_{7,6} = 7.6$ Hz, $J_{7,8} = 2.6$ Hz, H-7), 3.73 (1H, dd, $J_{5^*b,5} = 9.2$ Hz, H-5^{*b}), 4.3 (1H, ddd, $J_{5,6} = 2.5$ Hz, H-5), 4.36 (1H, dd, H-6), 4.5 (2H, AB spectrum, PhCH₂O-), 4.58 and 4.72 (2H, AB spectrum, $J_{gem} = 12$ Hz, PhCH₂O-), 4.69 and 4.74 (2H, AB spectrum, $J_{gem} = 11$ Hz, PhCH₂O-), 4.97 (1H, d, H-2), 7.2-7.33 (15H, m).

δ_C (125MHz): -4.9, -4.6, 18.2, 25.8, 62.5 (C-5), 64.5 (C-8), 70.8 (C-5^{*}), 72.2, 73.2, 73.4, 77.8 (C-6), 81.3 (C-7), 81.5 (C-2), 95.9 (C-3), 127.8, 127.9, 128, 128.4, 128.6, 137.6, 137.8, 138.1, 150.7 (C-8a).

Compound 13: To a solution of **12** (0.8 g, 0.956 mmol) in THF (10 ml) ethyl magnesium bromide solution in THF (1.3 ml, 1.3 mmol) was added at 0°C. After 10 min the reaction was quenched by addition of saturated ammonium chloride solution. The reaction was diluted with ethyl acetate and washed with brine. The aqueous layer was extracted once more with ethyl acetate. The combined organic layer was dried and evaporated. The residue was purified by flash column chromatography in PE-EA 15% to give 0.632 g (0.89 mmol, 93%) of the target product (R_f = 0.35 PE-EA 15%).

HRMS: Positive mode, m/z = 711.2088; expected for $C_{35}H_{44}IN_2O_4Si$ $[M+H]^+$ = 711.2115.

δ_H (500 MHz): 0.0 and 0.23 (6H, 2×s, -Si(CH₃)C(CH₃)₃), 0.89 (9H, s, -Si(CH₃)C(CH₃)₃), 3.5 (1H, dd, $J_{5a^*,5} = 7$ Hz, $J_{5a^*,5b^*} = 10$ Hz, H-5*a), 3.62 (1H, dd, $J_{5b^*,5} = 3$ Hz, H-5*b), 3.74 (1H, dd, $J_{7,8} = 2.3$ Hz, $J_{7,6} = 9.3$ Hz, H-7), 4.05 (1H, ddd, $J_{5,6} = 10$ Hz, H-5), 4.2 (1H, dd, H-6), 4.42 (2H, AB spectrum, PhCH₂O-), 4.62 and 4.92 (2H, AB spectrum, $J_{gem} = 11.6$ Hz, PhCH₂O-), 4.64 and 4.8 (2H, AB spectrum, $J_{gem} = 11.8$ Hz, PhCH₂O-), 5.07 (1H, d, H-8), 7.14 (1H, s, H-3), 7.19-7.5 (15H, m, 3×PhCH₂O-).

δ_C (125 MHz): -4.9, -4.5, 18.3, 25.9, 60 (C-5), 63.6 (C-8), 70.5 (C-5*), 71.8, 73.2 (C-6), 74.8, 80.9 (C-7), 82 (C-2), 124.5 (C-3), 127.83, 128, 128.5, 128.6, 137.4, 137.8, 137.9, 147.2 (C-8a).

Compound 14: A solution of **13** (0.315 g, 0.44 mmol), phenyl acetylene (0.243 ml, 2.22 mmol) and triethylamine (0.31 ml, 2.22 mmol) in DMF (5 ml) was degassed by freezing, evacuating and filling with argon three times. Cuprous iodide (0.01 g, 0.05 mmol) and Pd catalyst (0.058 g, 0.05 mmol) were then added to the degassed solution. The reaction was stirred for 16 h at +80°C. The reaction was concentrated in vacuum. The residue was dissolved in ethyl acetate and washed with water and brine. The aqueous layer was extracted with ethyl acetate once more. The combined organic layer was dried and evaporated. The brown residue was purified by flash column chromatography in PE-EA

15% to give 0.29 g (0.42 mmol, 96 %) of the target product as an amber coloured amorphous compound (R_f = 0.33 PE-EA 15%).

HRMS: Positive mode, m/z = 685.3408; expected for $C_{43}H_{49}N_2O_4Si$ $[M+H]^+ = 685.3462$.

δ_H (500 MHz): 0.0 and 0.22 (6H, 2 \times s, -Si(CH₃)C(CH₃)₃), 0.85 (9H, s, -Si(CH₃)C(CH₃)₃), 3.51 (1H, dd, $J_{5a^*,5} = 7.4$ Hz, $J_{5a^*,5b^*} = 10$ Hz, H-5*a), 3.62 (1H, dd, $J_{5b^*,5} = 3.1$ Hz, H-5*b), 3.76 (1H, dd, $J_{7,8} = 2$ Hz, $J_{7,6} = 9.1$ Hz, H-7), 4.07 (1H, ddd, $J_{5,6} = 10$ Hz, H-5), 4.21 (1H, dd, H-6), 4.43 (2H, AB spectrum, PhCH₂O-), 4.62 and 4.92 (2H, AB spectrum, $J_{gem} = 11.6$ Hz, PhCH₂O-), 4.65 and 4.8 (2H, AB spectrum, $J_{gem} = 11.8$ Hz, PhCH₂O-), 5.08 (1H, d, H-8), 7.06-7.43 (21H, m, 3 \times PhCH₂O-; PhC \equiv C- and H-3).

δ_C (125 MHz): -4.9, -4.5, 8.5, 18.2, 25.8, 45.6, 60.1 (C-5), 63.7 (C-8), 70.85 C-5*), 71.82, 73.22, 73.3 (C-6), 74.7, 81.1 (C-7), 83.3 (C \equiv C), 89.2 (C \equiv C), 123.2 (C-3), 123.4, 124, 127.7, 127.8, 127.9, 128, 128.3, 128.5, 128.6, 129, 130, 131.5, 137.5, 137.8, 137.9, 145.4 (C-8a).

Compound 15: A stock 1M solution of TBAF in THF (1 ml, 1 mmol) was added to a solution of **14** (0.289 g, 0.42 mmol) in THF (10 ml) at RT. The reaction was kept for 1 h at RT. The reaction was concentrated in vacuum. The residue was dissolved in DCM and washed with water. The aqueous layer was extracted with DCM once more. The combined organic layer was dried and evaporated. The brown residue was purified by flash column chromatography in Tol-EA 20 \rightarrow 25% to give 0.183 g (0.32 mmol, 76 %) of the target product as yellow amorphous compound (R_f = 0.2 Tol-EA 20%).

HRMS: Positive mode, m/z = 571.2590; expected for $C_{37}H_{35}N_2O_4$ $[M+H]^+ = 571.2597$.

δ_H (500 MHz): 3.62 (2H, m, H-5*a, H-5*b), 3.88 (1H, dd, $J = 3.2$ Hz, $J = 6.8$ Hz, H-7), 4.05 (2H, m, H-5, H-6), 4.25 and 4.34 (2H, AB spectrum, $J_{gem} = 12$ Hz, PhCH₂O-), 4.41 and 4.72 (2H, AB spectrum, $J_{gem} = 11.4$ Hz, PhCH₂O-), 4.61 and 4.77 (2H, AB spectrum, $J_{gem} = 12$ Hz, PhCH₂O-), 5.1 (1H, d, $J = 1.8$ Hz, H-8), 7.0-7.3 (19H, m), 7.38 (2H, m).

δ_C (125 MHz): 58.5 (C-5), 61.8 (C-8), 70 (C-5*), 70.6, 71.6, 72.1, 72.5 (C-6), 72.9, 77.2 (C-7), 82.1 (C \equiv C), 88.4 (C \equiv C), 121.6 (C-3), 122.26, 122.8, 124.3, 126.7, 126.8, 126.9, 127.2, 127.4, 127.7, 128, 130.4, 130.7, 136.3, 136.4, 136.7, 144.5 (C-8a).

Compound 16: To a solution of **15** (0.32 g, 0.56 mmol) in toluene (5ml) diphenylphosphoryl azide (0.614 ml, 2.85 mmol) was added followed by diazobicycloundecene (0.426 ml, 2.85 mmol) at RT. The reaction was kept for 4 h at RT. The reaction was diluted with THF to dissolve the oily sediment and heated-up to boil for 1min. The reaction was then concentrated in vacuum. The brown residue was purified by flash column chromatography in PE-EA 5 \rightarrow 25% to give 0.317 g (0.53 mmol, 92 %) of the target product (R_f = 0.43 PE-EA 20%).

$[\alpha]_D = +31.8^\circ$ c 1.0 CHCl₃

HRMS: Positive mode, m/z = 596.2627; expected for C₃₇H₃₄N₅O₃ $[M+H]^+ = 596.2662$.

δ_H (500 MHz): 3.58 (1H, dd, $J_{5a^*,5} = 5.2$ Hz, $J_{5a^*,5b^*} = 10.4$ Hz, H-5*a), 3.66 (1H, dd, $J_{5b^*,5} = 3$ Hz, H-5*b), 3.81 (1H, dd, $J_{7,8} = 7.1$ Hz, $J_{7,6} = 8.2$ Hz, H-7), 3.86 (1H, dd, $J_{6,5} = 7.5$ Hz, H-6), 4.05 (1H, ddd, H-5), 4.3 and 4.35 (2H, AB spectrum, $J_{gem} = 12$ Hz, PhCH₂O-), 4.44 and 4.78 (2H, AB spectrum, $J_{gem} = 11.2$ Hz, PhCH₂O-), 4.61 (1H, d, H-8), 4.71 and 4.78 (2H, AB spectrum, $J_{gem} = 11$ Hz, PhCH₂O-), 7.05-7.5 (21H, m, 3 \times PhCH₂O-; PhC \equiv C- and H-3).

δ_C (75 MHz): 58.9 (C-8), 59.1 (C-5), 68.5 (C-5*), 73.35, 74.6, 74.9, 75.1 (C-6), 80.6 (C-7), 82.9(C \equiv C), 89.6 (C \equiv C), 122 (C-3), 123.3, 125.24, 128, 128.1, 128.16, 128.2, 128.3, 128.6, 131.55, 137.1, 137.2, 141.4 (C-8a).

Compound 17: To a solution of **16** (0.12 g, 0.201 mmol) in THF-H₂O 10:1 (2 ml) triphenylphosphine (0.11 g, 0.42 mmol) was added at RT. The reaction was kept for 3 h at +60°C. The reaction was cooled down to RT and isobutyric anhydride (0.1 ml, 0.6 mmol) was added followed by triethylamine (0.2 ml, 1.4 mmol). The resulting turbid solution was kept RT for 1.5 h and then evaporated with toluene (2 \times 10ml). The residue

was purified by flash column chromatography in PE-EA 5→25% to give 0.12 g (0.19 mmol, 95 %) of the target product (R_f = 0.31 PE-EA 25%).

HRMS: Positive mode, m/z = 640.3168; expected for $C_{41}H_{42}N_3O_4$ $[M+H]^+ = 640.3175$.

δ_H (500 MHz): 0.99 and 1.03 (6H, 2×d, J = 7.8 Hz, $(CH_3)_2CH-$), 2.2 (1H, quint, $(CH_3)_2CH-$), 3.67 (2H, m, H-5*a, H-5*b), 3.93 (1H, dd, $J_{6,5} = 2.6$ Hz, $J_{6,7} = 4.7$ Hz, H-6), 4.05 (1H, dd, $J_{7,8} = 2.3$ Hz, H-7), 4.34 and 4.42 (2H, AB spectrum, $J_{gem} = 11.7$ Hz, $PhCH_2O-$), 4.4 (1H, m, H-5), 4.47 (2H, AB spectrum, $PhCH_2O-$), 4.62 and 4.83 (2H, AB spectrum, $J_{gem} = 11.7$ Hz, $PhCH_2O-$), 5.31 (1H, dd, $J_{8,NH} = 7.6$ Hz, H-8), 6.18 (1H, bd, $(CH_3)_2CHCONH-$), 7.09 (2H, m), 7.15-7.31 (17H, m), 7.44 (2H, m).

δ_C (125 MHz): 19.1 and 19.6 ($(CH_3)_2CH-$), 35.3 ($(CH_3)_2CH-$), 46.2 (C-8), 59 (C-5), 71.3 (C-5*), 72.5, 72.7, 73.5, 74.3 (C-6), 76.1 (C-7), 82.9 ($C\equiv C$), 89.6 ($C\equiv C$), 123.3 (C-2), 124.2, 127.7, 127.8, 127.9, 128, 128.3, 128.4, 128.6, 131.5, 136.8, 137.4, 137.6, 141.8 (C-8a), 176.1 ($(CH_3)_2CHCONH-$).

Compound 1: A solution of **17** (0.1 g, 0.16 mmol) in acetic acid (3 ml) was added to a slurry of $Pd(OH)_2/C$ (0.5 g) in acetic acid (2ml) preliminary activated by stirring under slight H_2 overpressure for 30 min at RT. The reaction flask was flushed with H_2 and the reaction was stirred under slight H_2 overpressure for 5h at RT. The reaction was filtered through a pad of Celite with the aid of MeOH and the filtrate was evaporated to dryness. The residue was purified by flash column chromatography in gradient $CHCl_3$ -MeOH 5→20% to give 0.036 g (0.096 mmol, 60 %) of the target product (R_f = 0.4 DCM-MeOH 30 %).

HRMS: Positive mode, m/z = 374.2075; expected for $C_{20}H_{28}N_3O_4$ $[M+H]^+ = 374.2080$.

δ_H (500 MHz, Pyridine d_5): 1.18 and 1.19 (6H, 2×d, J = 6.8 Hz, $(CH_3)_2CH-$), 2.66 (1H, quint, $(CH_3)_2CH-$), 2.95 (4H, m, $-CH_2CH_2-$), 4.2 (1H, m, H-5), 4.3 (2H, m, H-5*a, H-7), 4.47 (1H, dd, $J_{6,5} = 8.6$ Hz, $J_{6,7} = 8.6$ Hz, H-6), 4.55 (1H, dd, $J_{5*a,5*b} = 11.7$ Hz, $J_{5*b,6} =$

2.6 Hz, H-5*b), 5.73 (1H, dd, $J_{8,7} = 8.4$ Hz, $J_{8,NH} = 8.4$ Hz, H-8), 7.1 (1H, m), 7.17-7.2 (4H, 2×s), 7.28 (1H, bs, H-3), 8.81 (1H, bd, (CH₃)₂CHCONH-),

δ_C (125 MHz): 21.9 and 22.1 ((CH₃)₂CH-), 33.3 (-CH₂-), 37.7 (CH₃)₂CH-, 38.3 (-CH₂-), 53.7 (C-8), 64.1 (C-5*), 64.8 (C-5), 72.3 (C-6), 77.6 (C-7), 116.5 (C-3), 128.1, 130.7, 130.8, 144.4, 144.9, 146.4, 180.1.

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